

In re Application of:  
Nelson et al.  
Application No.: 09/687,246  
Filed: October 12, 2000  
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PATENT  
Atty Docket No.: JHU1660-1

Exhibit A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Twice amended) A method for detecting a hepatic cell proliferative disorder in a human, comprising: detecting a methylated CpG-containing glutathione-S-transferase (GST) nucleic acid in a hepatic specimen or biological fluid wherein a methylated GST nucleic acid is indicative of a hepatic cell proliferative disorder.
3. (Amended) The method of claim 2, wherein the primers flank a region in the promoter of GST, wherein said promoter contains a transcriptional start site for GST.
4. (Twice amended) The method of claim 3, wherein the promoter region is approximately -539 to -239 upstream from the transcriptional start site.
6. (Amended) The method of claim 1, wherein the detecting comprises contacting a nucleic acid-containing hepatic specimen or biological fluid with an agent that modifies unmethylated cytosine, amplifying the CpG-containing nucleic acid in the specimen by means of CpG-specific oligonucleotide primers, wherein the oligonucleotide primers distinguish between modified methylated and nonmethylated nucleic acid, and detecting the methylated CpG-containing GST nucleic acid based on the presence or absence of amplification products produced in said amplifying step.
8. (Amended) The method of claim 6, wherein the [oligonucleotide] oligonucleotide primers have a sequence [as set forth in] selected from the group consisting of SEQ ID NO: 7, 8, 9, 10, 11, 12, or 13,
11. (Amended) The method of claim 1, wherein the CpG-containing GST nucleic acid is a promoter region.

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16. (Amended) The method of claim 1, wherein the detecting is by contacting a target nucleic acid in the hepatic specimen or biological fluid with a reagent which detects methylation of the promoter region of the GST nucleic acid when the target nucleic acid is DNA, or wherein the reagent detects the level of GST RNA when the target nucleic acid is RNA; and detecting the GST [target] nucleic acid, wherein hypermethylation of the promoter of GST DNA, or decreased levels of GST RNA, as compared with the level of GST RNA in a normal cell, is indicative of a GST-associated cell proliferative disorder in hepatic tissue.
18. (Amended) The method of claim 1, wherein the GST nucleic acid is a  $\pi$  family GST nucleic acid.
20. (Amended) The method of claim 16 wherein the reagent which detects methylation of the promoter region of the GST nucleic acid is a restriction endonuclease.
27. (Amended) The method of claim 1, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.
39. (Amended) The method of claim 28, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.
41. (Amended) The method of claim 40, wherein the GST nucleic acid is DNA.
42. (Amended) The method of claim 40 wherein the GST nucleic acid is RNA.
51. (Amended) The method of claim 40, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.

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84. (Amended) The method as in any of claims 1, 28, or 40[, or 52], wherein methylation is in one allele.

85. (Amended) The method as in any of claims 1, 28, or 40[, or 52], wherein methylation is in both alleles.